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THE RELATION OF THE NYMPHS OF A DRAGON FLY (*Libellula pulchella* DRURY) TO ACID AND TEMPERATURE[1]

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Materials and Methods

The dragon-fly nymphs were collected in a quiet muddy pocket of a badly polluted small stream called the "Boneyard," which flows through Champaign-Urbana, Illinois. All the nymphs were found buried in mud at a depth of one and a half feet from the surface of the water. Collections were made on two different occasions—in the fall on October 3 and in the spring on April 24. On both occasions this quiet muddy pocket contained a considerable number of the nymphs about fully developed. The mud in which they were living on April 24 was acid, pH 6.4, and the surface of the water only slightly less so, pH 6.6. This is a higher degree of acidity than is usually the case. Most streams are appreciably alkaline. No stream has been recorded, so far as I know, that normally showed a hydrogen-ion concentration greater than 5.8. Very few nymphs were found anywhere else in the stream, and then only in mud under debris such as pieces of wood and tin cans.

In my experiments I used a box of thin sheet iron, about two feet long, one and a half inches wide, and one and a half inches deep. A vertical row of holes was punched in the middle of the box on each side to let out the water. An acid solution was produced by mixing water and acid in a Mason jar in such proportions that the desired pH values could be obtained. The water used throughout was aerated University of Illinois water; the acid was hydrochloric. Except in experiments where special temperatures were used, the range was from 18°–24° C., generally around 20° C. In order to insure uniformity of light, two 50-watt Mazda lamps were placed immediately above and at equal distances from the ends of the box. The pH determinations were made by the colorimetric method, using standard buffer solutions at the lower concentrations, and McClendon's color chart for concentrations greater than pH 4.0. The nymphs, until used in the experiments, were kept in shallow jars containing gravelly mud and rotting leaves, with some two inches of water. The jars were immersed in cool running water of an average temperature of 20° C. Since the nymphs are cannibalistic, they were not fed. From time to time as their water became foul they were given a clean supply.

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[1] Contribution from the Zoological Laboratories, University of Illinois, No. 203.

Experimental Results

Reactions

Acid gradients.—To produce an acid gradient, the acid solution from the Mason jar was allowed to flow in at one end of the box, and unmodified water was allowed to flow in at the other end at the same rate. The two liquids mixed sufficiently to produce a definite gradient, and flowed out through the row of holes in the middle of the box. At first a slight acidity was used, ranging from pH 6.4 to 8.4. The results are given in Table I.

Table I. *Response of dragon-fly nymphs to slight acidity and alkalinity*

Readings every two minutes (after the first five minutes). Every 5th reading

Shown	Acid			Neutral		Alkaline		
pH	6.4	6.4	6.6	7.1		8.2	8.4	8.4
1st	4	2	2	0	4	2	1	5
5th	5	0	2	2	2	2	2	5
10th	4	2	0	2	0	2	3	6
15th	2	2	2	0	4	2	0	8
20th	1	5	2	1	3	0	2	6
25th	3	1	1	0	2	3	1	9
Total all readings	82 16%	47 9%	34 7%	36 7%	57 11%	39 8%	36 7%	167 33%

Rate of flow—55 c.c. per minute.

Temperature—Acid end, 21°.5 C.; alkaline end, 22°.5 C.

Stock, in laboratory three months. Number of individuals, twenty.

Note. The 8 columns in this and the following tables represent 8 sections of the experimental box, and the numbers in the columns represent the number of nymphs in each section at each reading.

It will be seen that some preference was shown for the alkaline end, about twice as many nymphs congregating in this end as in the other. But it will also be noticed that considerably more nymphs collected at the acid end than anywhere else except the alkaline end. This "bunching" at either end was characteristic throughout the acid-gradient experiments. Subsequently stronger acidities were used.

Table II illustrates the reaction that was typical of a number of experiments in each of which the range of pH values was largely the same. The "bunching" of the nymphs was, however, not nearly so marked in this particular experiment as was generally the case. In this and subsequent tables the rate of flow was 26 c.c. per minute.

Table II. *Response to acidity and alkalinity*

Readings every three minutes (after the first five minutes)

pH 5.0	–	5.8	7.3		8.4	–	8.6
2......	0	4	5	2	2	3	2
2......	1	3	5	3	3	1	2
4......	1	1	6	2	1	2	3
4......	1	1	6	2	1	1	4
4......	1	2	3	3	1	2	4
4......	2	2	2	0	4	1	3
5......	1	2	3	0	4	2	3
3......	2	2	5	0	3	2	3
28......	9	17	34	12	19	14	25
18%....	6%	11%	21%	7%	12%	9%	16%

Temperature—Both ends, 18°.0 C.
Stock, in laboratory three months. Number of individuals, twenty.

An example of the effect of a marked acidity is shown in Table III. It will be observed that a very decided "bunching" took place at either end.

Table III. *Response to strong acidity and slight alkalinity*

pH	2.5	–	5.5	–	6.2	–	–	7.8	
	7	0	0	1	1	0	1	10	after one hour.
	7	0	0	1	2	0	0	10	after three hours.

Temperature—Both ends, 22°.5 C.
Stock, in laboratory ten days. Number of individuals, twenty.

At the conclusion of this experiment the seven individuals in the acid end were placed in a separate jar. After a week only one was dead. After eighteen days four had been attacked by fungi and were dead, but the remaining two were alive and vigorous. The individuals not in the acid end did not show any fungus infection. The results shown in Table III were corroborated a number of times in experiments in which the range of acidities employed were practically the same.

Table IV represents an experiment in which a pH of 1.0 was reached one fourth down the box. At the time I had no way of determining stronger acidities, so the concentration in the acid end of the box remained unknown, though it must have been considerably greater than pH 1.0. The nine individuals in this end were placed in a jar to themselves, and after a week all but one were living and apparently vigorous.

Table IV. *Response to very strong and moderate acidity*

pH	–	–	1.0	2.0		6.0	–	–	
	8	0	0	1	2	1	2	2	after one and a half hours.
	7	0	0	2	0	1	3	2	after two hours and ten minutes.

Temperature—Both ends, 24°.0 C.
Stock, in laboratory four weeks. Number of individuals, sixteen.

Uniform acidity.—The acid solution only was allowed to flow into the box, at the usual rate of 26 c.c. per minute, and had to be continuous to produce a constant pH value. Out of eight individuals, from stock in the laboratory four weeks, in a uniform acidity of pH 1.0, two were at one end and six at the other. This shows that the nymphs can stand a constant pH of 1.0 at a temperature of 21° C. for twelve and a half hours. At the end of this time they were all alive and apparently vigorous.

UNIFORM ACIDITY WITH HIGH TEMPERATURE

In an experiment in which the pH was uniformly 1.0 and the temperature was 34°.5 C., six nymphs showed distinct irritability immediately on being placed in the experimental box. They showed no inclination to bunch at any time. At the end of an hour one was dead. Two hours later there was no further change. Eleven hours later the acid tube was found clogged and the pH was about 7.0, but the temperature was the same, 34°.5 C. All the individuals were alive. Considerable of the acid had been used, enough for four or five hours at a pH 1.0 flow. The pH was adjusted back to 1.0, and after four hours, or eighteen hours from the beginning of the experiment, three of the nymphs were still alive, and were living three days later. With a pH of 1.0 and the temperature raised to 40°.5 C., twelve individuals showed, as in previous experiments, the same distinct irritability with no tendency to bunch, and were all dead in forty-five minutes.

INCREASED TEMPERATURES ALONE

No acid solution was used in this experiment, and the water, that of the University, was alkaline, pH 8.0. Five out of six nymphs appeared unharmed at a temperature of 37°.5–38°.0 C. for eighteen hours. Five fresh individuals were placed in the box with a temperature of 40°.0–41°.5 C. Two nymphs were dead after eight hours. Thirteen and a half hours later another was dead, and the two remaining were nearly dead. At a temperature of 42°.5 C. six fresh individuals were all alive after one hour, but all dead after four hours and ten minutes. At 44°.0 C., of four individuals, one was dead in eleven minutes, another in 25 minutes, and the remaining two in one hour and five minutes. At 45°.0–46°.0 C. the nymphs will live as long as nineteen minutes. At 50°.5 C. they will live only a few seconds.

Conclusions

The outstanding result of these experiments is the great hardiness of the nymphs of *Libellula pulchella.* They lived very well for more than six months in alternating foul and fresh water. They lived apparently unharmed in water as strongly acid as pH 1.0, with more or less indifference to the degree of acidity present. The literature I know of that deals in pH values seems to agree that the animals experimented on succumb to a pH of 5.0, and select an environment with a reaction around neutrality. Of nine species of fish, the selection ranged from pH 6.8 to 8.0. Furthermore, the nymphs of *Libellula pulchella* are apparently uninjured by a temperature distinctly greater than that of mammalian blood.

The foregoing experiments were carried on under the supervision of Prof. V. E. Shelford. Sincere thanks are extended to him for many helpful suggestions offered during the progress of the work.

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